Cascade screening for Familial Hypercholesterolemia in South Africa reveals a significant number of subjects with more than one FH mutation: The Wits FIND-FH Program

Frederick J Raal[1], El Mustapha Bahassi[2], Belinda Stevens[1], Traci A Turner[2] and Evan A Stein[2].

[1]Stein Center for FH, Carbohydrate and Lipid Metabolism Research Unit, Department of Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. South Africa;
[2]Medpace and Medpace Reference Laboratories (MRL), Cincinnati. Ohio. USA.

Background

- **Familial hypercholesterolemia** (FH) is an autosomal co-dominant disorder usually resulting from mutations in the LDL receptor (LDLR) gene and less commonly from mutations in apoB100, PCSK9 or LDLRAP1.

  This condition is characterized by elevated levels of LDL-cholesterol (LDL-C) and premature cardiovascular disease, particularly coronary artery disease (CAD)[1].
- The heterozygous phenotype (HeFH) is characterized by elevated LDL-C levels approximately twice the normal levels (4.9 – 10 mmol/L), tendon xanthoma and premature CAD. If untreated, the cumulative risk of a coronary event by the age of 60 years is at >50% in men and 30% in women. The homozygous phenotype (HoFH) is characterized by LDL-C levels >13 mmol/L, skin and tendon xanthoma beginning soon after birth and if untreated CAD prior to age 20 years of age. There is however some overlap in the clinical phenotype between HeFH and HoFH when assessed by genotype.[2]
- FH is one of the commonest inherited diseases in the world with an estimated frequency of 1:200 to 1:250 for Caucasian populations[1]. However in some countries such as South Africa, the prevalence in certain population groups such as the Afrikaner, Jewish and south-Asian Indians it is as high as 1:80probably due to a founder effect. [3,4]
- In South Africa 70 to 80% of subjects of Afrikaner, Jewish or Indian origin with clinical heterozygous FH identified to date have one of 5 founder mutations - table 1. However the vast majority of FH patients remain undiagnosed and untreated and have not been screened for other mutations in the LDLR, ApoB, PCSK9 or LDLRAP1 genes.

TABLE 1: Founder FH LDLR mutations common in South Africa

Location on LDLR gene	Codon Change	Amino acid change	Common name	Population group
Exon 4	NM_000527.4:C.681c>G	D227E	FH Afrikaner 1	Afrikaner*
Exon 9	NM_000527.4:C.1285G>A	V429M	FH Afrikaner 2	Afrikaner*
Exon 4	NM_000527.4:c523G>A	D154N	FH Afrikaner 3	Afrikaner*
	NM_000527.4:c.2054C>T	P664L	FH Gujarat	South Asian
Exon 14	NM_000527.4:c.654_656deTGG	GLY219del	FH Lithuania	Lithuanian

* Includes both Caucasian and SA 'coloured or mixed' populations

- Until recently no systematic program existed to detect subjects with FH or to test their family members. Furthermore, information regarding prevalence of FH in black South Africans is sparse. The Wits FIND-FH program was initiated in late 2016 with the goal of addressing both these issues.

Methods

- After obtaining an IRB approved written informed consent from a known FH index case, 1st degree relatives were contacted and a home or clinic visit arranged where after individual informed consent was obtained a targeted medical, cardiovascular, family and medication history, physical (including for skin and tendon xanthoma and corneal arcus) and blood sample were obtained. Fasting blood samples obtained from subjects were analyzed at Medpace Reference Laboratories (MRL), Leuven, Belgium for lipids and apolipoproteins and select chemistries to exclude underlying metabolic conditions known to cause secondary elevations of LDL-C.
- In patients with likely FH by clinical assessment, DNA analysis for LDLR, APOB, PCSK9, LDLRAP1 mutations were analyzed by Next Generation Sequencing (NGS). Briefly whole blood samples were drawn into a K2EDTA tube and Initially processed at MRL Belgium and genomic DNA isolated using the QIAamp DNA Blood kit (Germantown, MD).
- The genomic DNA (gDNA) was sequenced at MRL in Cincinnati, Ohio. Sequencing was performed for the coding regions of four genes (LDLR, APOB, PCSK9, LDLRAP1) known to account for the majority of cases of FH.5ApoB exons 1-29, LDLR exons 1-18, LDLRAP1 exons 1-9 and PCSK9 exons 1-12 were captured, amplified by PCR and subjected to bidirectional DNA sequencing using the Illumina Mi Sequencing platform(San Diego, CA). The sequencing probes were designed based on the GRch37/hg19 reference genome. Secondary and tertiary analysis of DNA sequences were performed using commercial bioinformatics software.
- The bioinformatics method used also identifies DNA copy number variations (CNVs) in the LDLR gene which are the cause of FH in up to 10% of cases. Variants identified were compared to the GRCh37/hg19 reference genome and the pathogenicity of reported variants were determined according to current guidelines.[6]

Results

One full-time and 1 part-time research nurse were hired and trained in late 2016 and beginning in January 2017 follow up of family members commenced based on index patients identified from the Wits Lipid Clinic. To date 700 subjects have been screened with 479 clinically diagnosed as probable or definite FH using the Simon Broome criteria. Demographic and LDL-C levels are shown in table 2. Genetic analysis confirmed 285/479 (59.5%) as having mutations consistent with FH –table 3. CNVs in the LDLR gene were found in 16 of these subjects (5.6%). The program has identified a small but growing number of black South Africans with FH, including 2 subjects with genetically confirmed homozygous FH - table 4.

TABLE 2

	Variant identified				
	Total (285)	**Male (129)**	**Female (156)**	**Treated (182)**	**Untreated (103)**
LDL-C (mmol/L)	4.9 ± 2.5	4.8 ± 2.2	5.0 ± 2.6	4.3 ± 2.0	6.0 ± 2.7
Apolipoprotein B (mg/dL)	135 ± 47	133 ± 43	137 ± 50	125 ± 41	153 ± 52
Lipoprotein (a) (nmol/L)	64 (22-152)	56 (20-147)	71 (24-162)	83 (22-167)	45 (21-121)
	No variant identified				
	Total (194)	**Male (94)**	**Female (100)**	**Treated (91)**	**Untreated (103)**
LDL-C (mmol/L)	3.6 ± 1.2	3.5 ± 1.3	3.7 ± 1.2	3.0 ± 1.1	4.2 ± 1.1
Apolipoprotein B (mg/dL)	113 ± 31	113 ± 31	113 ± 32	104 ± 28	121 ± 31
Lipoprotein (a) (nmol/L)	39 (15-151)	29 (11-106)	53 (20-166)	57 (18-171)	32 (11-95)

LDL-C and Apolipoprotein B presented as mean ± SD. Lipoprotein (a) as median (IQR)

TABLE 3

Race	Mutation +ve	Mutation -ve	Spectrum of common FH causing mutations
White (332)	211 (64%)	121 (36%)	**LDR mutations (n=187; 89%)** FH Afrikaner-1 (c.681C>G = 73 FH Afrikaner-2 (c.1285G>A) = 50 } 130 (63%) FH Afrikaner-3 (c.523G>A) = 7 **ApoB mutations (n=22; 10%)** **PCSK9 mutations (n=2; 1%)**
Indian (115)	52 (45%)	63 (55%)	**LDR mutations (n=44; 85%)** FH Gujarat (c.2054C>T = 30 (68%) **ApoB mutations (n=7; 13%)** **PCSK9 mutations (n=1; 2%)**
Mixed Race (16)	10 (63%)	6 (37%)	**LDR mutations (n=7; 70%)** FH Afrikaner-2 (c.1285G>T = 4 **ApoB mutations (n=1; 10%)** **PCSK9 mutations (n=67%)**
Black African (16)	12 (63%)	6 (37%)	**LDR mutations (n=8; 67%)** FH Cape Town (c.137-142 del) = 3 **ApoB mutations (3, 25%)** **PCSK9 mutations (n=1; 8%)**

TABLE 4 – Black African subjects with FH

NUMBER	AGE	UNTREATED LDL (mmol/L)	Clinical markers of FH	CVD/AGE ONSET	MUTATIONS found on NGS	FH GENE VARIANT	PATH
1	80	5.3	arcus	Nil	-	-	NEGATIVE
2	43	5.2	arcus,tendons	Nil	NM_000527.4:c.del137-142	LDLR	PATH
3	**25**	**11.1**	**arcus, tendons, xanthelasma**	**Nil**	**NM_000527.4:c.del137-142 NM_000527.4:c.del137-142**	**LDLR**	**PATH PATH ***
4	82	8.2	arcus, tendon	TIA age 71	NM_000527.4:c.del137-142	LDLR	PATH
5	79	11.4	arcus, tendon	Nil	NM_000527.4:c.1222G>A NM_000527.4:c.1104C>T	LDLR	PATH UNCERT
6	55	9.5	arcus, tendons, other	Nil	NM_000527.4:c.313+1G>A NM_000527.4:c.757C>T	LDLR	PATH UNCERT
7	57	4.3	arcus	Nil	-	-	NEGATIVE
8	55	11.5	arcus, tendon, xanthelasma	Nil	NM_000383.2:c.7501A>T	APOB	UNCERT
9	70	9.4	arcus	Nil	NM_000384.2:c.8889C>T NM_000384.2:c.7242A>C	APOB	UNCERT UNCERT
10	52	7.4	arcus, tendon	Nil	NM_000527.4:c.414G>C	LDLR	PATH
11	28	5.2	tendon	Nil	NM_000527.4:c.1285G>A	LDLR	PATH
12	**86**	**9**	**arcus, tendon, planar**	**Nil**	**NM_000527.4:c.829G>A NM_000527.4:c. 2441G>A**	**LDLR**	**PATH PATH ***
13	48	4.7	NO	Nil	NM_174936.3:c.1658A>G	PCSK9	UNCERT
14	50	6.7	NO	Nil	-	-	NEGATIVE
15	27	3.7	arcus, tendon	Nil	-	-	NEGATIVE
16	45	5.2	NO	Nil	NM_000384.2:c.G>A	APOB	UNCERT

NM_000527.4:c = LDLR mutation
NM_000383/4.2:c = ApoB mutation
NM_174936.3:c = PCSK9 mutation
* = genetically confirmed homoygous FH

TABLE 5 – Subjects with two or more FH gene mutations

NUMBER	ETHNICITY	AGE (years)	UNTREATED LDL (mmol/L)	Clinical markers of FH	CVD/AGE ONSET	Mutations found on NGS	FH GENE VARIANT	PATH
1	**W**	**9**	**12.3**	**tendon,arcus, planar**		**NM_000527.4:c.681C>G NM_527.4:C.1285G>A**	**LDLR/LDLR**	**PATH X 2***
2	**BA**	**25**	**11.1**	**tendon,arcus, planar**		**NM_000527.4:c.137_142del x 2**	**LDLR/LDLR**	**PATH X 2***
3	**I**	**13**	**18.6**	**tendon,arcus,planar**		**NM_000527.4:c.2054C>T NM_000527.4:c.401G>A**	**LDLR/LDLR**	**PATH X 2***
4	**W**	**3**	**25.4**	**tendon,planar**		**NM_000527.4:c.1285G>A NM_000527.4:c.1285G>A**	**LDLR/LDLR**	**PATH X 2***
5	**W**	**12**	**11.5**	**tendon, planar**		**NM_000527.4:c.1285G>A NM_000527.4:c.1285G>A**	**LDLR/LDLR**	**PATH X 2***
6	W	76	7.2	arcus, tendon	CABG age 46	NM_000527.4:c.681C>G Duplication exons 1-2	LDLR/LDLR	PATH X 2
7	W	22	5	tendon		NM_000527.4:c.681C>G Deletions exons1-3, 7-11, 13-18	LDLR/LDLR	PATH X 4
8	W	49	4.5	tendon		NM_000527.4:c.681C>G deletion exons 91-19 duplication exons 1-2	LDLR/LDLR	PATH X 3
9	W	68	4	tendon		NM_000527.4:c.681C>G Duplication exons 1-2	LDLR/LDLR	PATH X 2
10	BA	79	8.1	arcus, tendon		NM_000527.4:c.1222g>a NM_000527.4:c.1104C>T	LDLR/LDLR	PATH X 2
11	I	43	7.4	arcus, tendon, planar, other	CABG age 29 and 42	NM_000527.4:c.268G>T NM_000527.4:c.1951G>A	LDLR/LDLR	PATH X 2
12	I	50	6.9	tendon	MI age 42/CABG age 46	NM_000527.4:c.1724T>G NM_000527.4:c.649C>T	LDLR/LDLR	PATH X 2
13	I	45	9.5	arcus/tendon		NM_000527.4:c.1724T>G NM_000527.4:c.649C>T	LDLR/LDLR	PATH X 2
14	W	67	4.2	nil		NM_000527.4:c.2359G>A deletion exons 13-15	LDLR/LDLR	PATH X 2
15	BA	86	9	arcus, tendon, planar		NM_000527.4:c.829G>A NM_000527.4:c.2441G>A	LDLR/LDLR	PATH X 2
16	W	32	9.3	tendon		NM_000527.4:c.662A>G NM_000527.4:c.2359G>A	LDLR/LDLR	PATH/UNCERT
17	I	9	6.7	nil		NM_000527.4:c.2054C>T NM_000527.4:c.2478C>G	LDLR/LDLR	PATH/UNCERT
18	I	60	11.5	arcus, tendon, planar		Deletion exons 9-10 NM_174863.3:c.1563C>T	LDLR/LDLR	PATH/UNCERT
19	BA	55	4.7	arcus, tendon, other		NM_000527.4:c.313+1G>A NM_000527.4:c.757C>T	LDLR/LDLR	PATH/UNCERT
20	W	30	6.4	nil		NM_000527.4:c.681C>G NM_000384.2:c.3383G>A	LDLR/APOB	PATH x 2
21	W	53	8.1	tendon	PCI age 44	NM_000527.4:c.681C>G NM_000384.2:c.C5599C>T	LDLR/APOB	PATH x 2
22	W	23	6.1	tendon		NM_000527.4:c.681C>G NM_000384.2:c.C5599C>T	LDLR/APOB	PATH x 2
23	W	56	8.7	tendon	PCI age 47	NM_000527.4:c.681C>G NM_000384.2:c.C5599C>T	LDLR/APOB	PATH x 2
24	A	64	5.9	arcus, tendon	CABG age 35	NM_000527.4:C2043C>A NM_000384.2:c.3927T>A NM_000384.2:C5254G>A	LDLR/APOB/APOB	PATH/UNCERT X 2
25	W	62	9.2	xanthelasma, arcus	CABG age 31	NM_000527.4:c.1048C>T NM_00384.2:c.152A>G	LDLR/APOB	PATH/UNCERT
26	W	55	8.6	arcus, tendon		NM_000527.4:c.1285G>A NM_000384.2:c.3383G>A	LDLR/APOB	PATH/UNCERT
27	W	29	7.4	nil		NM_000527.4:c.1285G>A NM_000384.2:c.3383G>A	LDLR/APOB	PATH/UNCERT
28	W	33	7.6	nil		NM_000527.4:c.1285G>A NM_000384.2:c.3383G>A	LDLR/APOB	PATH/UNCERT
29	I	13	12.9	nil		NM_000527.4:c.2054C>T NM_000384.2:c.8227C>A	LDLR/APOB	PATH/UNCERT
30	W	40	5.2	tendon		NM_000527.4:c.1285G>A NM_000384.2:c.3383G>A	LDLR/APOB	PATH/UNCERT
31	I	22	6	nil		NM_000527.4:c.2054C>T NM_000384.2:c.8227C>A	LDLR/APOB	PATH/UNCERT
32	W	61	7.3	arcus		NM_000527.4:c.682C>G NM_000384.2:c.3383G>A	LDLR/APOB	PATH/UNCERT
33	W	44	7.2	nil		Duplication exons 1-2 NM_000384.2:c.10131G>A	LDLR/APOB	PATH/UNCERT
34	W	44	5.2	nil		Deletion exons 12-14 NM_000384.2:c.3426G>A	LDLR/APOB	PATH/UNCERT
35	A	49	4.7	nil		NM_000527.4:c.1104C>T NM_174936.3:c.267G>A	LDLR/PCSK9	UNCERT X 2
36	W	45	6.1	nil		NM_000527.4:c.2043C>A NM_015627.2:c.284G>A	LDLR/LDLRAP1	PATH/UNCERT
37	W	50	4.8	nil		NM_000384.2:c.12382G>A NM_000384.2:c.10131G>A	APOB/APOB	UNCERT X 2

NM_000527.4:c = LDLR mutation
NM_000384.2:c = ApoB mutation
NM_015627.2:c = LDLRAP1 mutation
NM_174863.3:c = PCSK9 mutation
* = Clinical homozygous FH
PCI = per cutaneous coronary artery intervention
CABG = coronary artery bypass graft surgery
MI = myocardial infarction

Subjects with two or more FH gene mutations:

Five subjects met the clinical diagnosis for homozygous FH but DNA analysis revealed a further 32 patients, including 4 black African subjects, with two or more FH mutations. HoFH based on either a clinical or genetic diagnosis was therefore found in 37 (7.7%) of subjects who underwent DNA testing - table 5. Nineteen of these subjects (48%) has two mutations which are considered definitely pathogenic and the remainder has one or more mutations of uncertain pathogenicity.

Discussion

- Using phenotype cascade screening The Wits FIND-FH program has averaged 30 subjects monthly, found a clinical FH diagnosis in 68% of whom ≈ 60% were genetically confirmed. In addition, a number of genetic HoFH or compound HeFH patients who do not meet the traditional clinical criteria, especially LDL-C, for HoFH have been found.[2]
- The South African multi-ethnic society and well described founder effects emphasize the need for differential approaches to diagnosis and management of FH. Cascade testing of index cases based on phenotype is an important start and has identified many family members who were previously unaware that they had FH. The program is identifying a small but growing number of black South Africans with FH. While the prevalence of FH in virtually all populations in the world demonstrates a gene frequency of 1 per 200 to 500 there are no similar studies in black Africans where even case reports of FH in the literature are few. Studies involving larger cohorts and inclusive of different ethnicities are paramount to establishing an accurate prevalence of FH in black South Africans.

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